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APPLICATION NUMBER: *60/501,660*
FILING DATE: *September 09, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/25026*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
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PTO/SB/16 (08-03)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL 961801225 US

PTO
09/09/03
60/501660
17487 U.S.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Dusan	Miljkovic	San Diego, CA
Zbigniew	Pietrzkowsk	San Diego, CA

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

New Nutracetical Chromium Complexes with Enhanced Biological Activity and Safety

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

[X] Customer Number: 34284 — 34284

OR

[ ] Firm or Individual Name					
Address					
Address					
City		State		ZIP	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS ***(check all that apply)***

[X] Specification *Number of Pages* 11 — [ ] CD(s), Number _____

[ ] Drawing(s) *Number of Sheets* _____ — [ ] Other (specify) _____

[ ] Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[X] Applicant claims small entity status. See 37 CFR 1.27.

[ ] A check or money order is enclosed to cover the filing fees.

[X] The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 502191

[ ] Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($)

80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____ Date 9/9/03

TYPED or PRINTED NAME Martin Fessenmaier — REGISTRATION NO. 46697 *(If appropriate)*

TELEPHONE 714-641-5100 — Docket Number: 100700.0028PRO

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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FEE TRANSMITTAL for FY 2003

Effective 01/01/2003. Patent fees are subject to annual revision.

[X] Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT	($) 80.00

Complete if Known	
Application Number	
Filing Date	August 26, 2003
First Named Inventor	Dusan Miljkovic
Examiner Name	
Art Unit	
Attorney Docket No.	100700.0028PRO

METHOD OF PAYMENT *(check all that apply)*

[ ] Check [ ] Credit card [ ] Money Order [ ] Other [ ] None

[X] Deposit Account:

Deposit Account Number	502191
Deposit Account Name	Rutan & Tucker

The Commissioner is authorized to: *(check all that apply)*

[X] Charge fee(s) indicated below [X] Credit any overpayments

[X] Charge any additional fee(s) during the pendency of this application

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

		Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X	=	
Independent Claims		- 3** =		X	=	
Multiple Dependent					=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,300	2453	650	Petition to revive - unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($)

SUBMITTED BY

(Complete *(if applicable)*)

Name (Print/Type)	Martin Pessenmaier	Registration No. (Attorney/Agent)	46697	Telephone	714-641-5100
Signature				Date	September 9, 2003

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NEW NUTRACETICAL CHROMIUM COMLEXES WITH ENHANCED BILOGICAL ACTIVITY AND SAFETY

BACKGROUND

Numerous chromium nutraceutical complexes have been described so far in patent and scientific literature. These also include different chromium salts that need not to be real chromium complexes, indeed they may be week chromium complexes, or simple ionic salts, such as chromium chloride hexahydrate. Only Cr (3+) species are acceptable as nutraceuticals, since all other valence states of Cr are considered unsafe or human health, while Cr (6+) species is highly toxic/genotoxic.

Many chromium (III)-salts represent week chromium complexes, such as chromium salts with organic bi-tri-and poly-carboxylic acid (such as malic, tartaric and citric acid).

In spite of the relatively large number of known chromium salts and complexes, only few of them have been marketed. The most popular ones are chromium picolinate, and chromium polynicotinate (chromium niacin). Some other Cr (III) complexes, such as Cr-tri-carnosinate are also described in patent literature and are marketed.
In addition to those, drum-dried or spray-dried yeast grown in the presence of Cr (III) has been marketed used as a nutraceutical (known as "chromium yeast").

Although there are numerous publications that illustrate efficiency of the marketed chromium supplements to at least some degree, there are also a number of scientific reports where side effects and/or insufficient biological activity have been documented. Several articles describe appreciable genotoxicity of Chromium picolinate (especially in the presence of ascorbic acid that is normally found in all mammalian cells in appreciable concentration.

Very often water insolubility of the used Cr supplements (Cr picolinate, Cr polynicotinate, chromium yeast) is a serious drawback in their application (diminishing their biological activity/bioavailability and/or preventing researchers from testing them in cell culture systems in order to elucidate their biological mechanism of action).

Therefore, there is a constant need to find new chromium compounds/complexes that have higher biological activity/bioavailability, higher safety/less toxicity, sufficient chemical stability and high water solubility.

OUR INVENTION

Our invention relates to new nutraceutical chromium compounds/complexes with enhanced biological activity and safety, and their use. More specifically, through our extended chemical and biological research, we unexpectedly discovered that the optimal (maximal) biological activity of Cr(III) species depend on the chemical stability of their complexes with different ligands directly measured through their association constants or

indirectly measured through the position of their maxima in their visible spectrum (lambda $_{max}$).

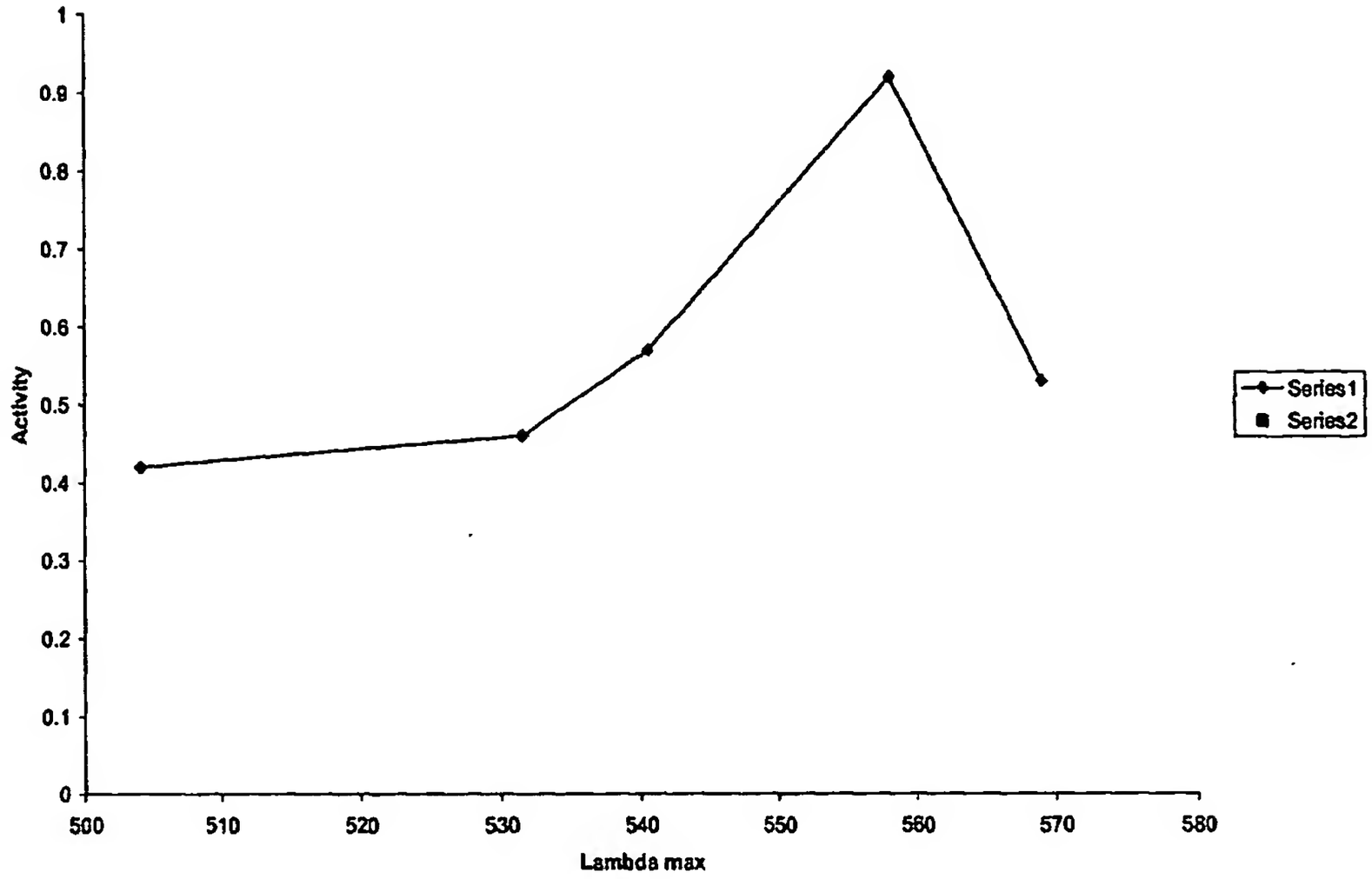
Particularly striking examples include different Cr complexes with dipeptide carnosine. CRC5 (chromium-penta-carnosinate), CRC3R (chromium-tri-carnosinate-red) and CRC3V (chromium tri-carnosinate-violet) are the chromium complexes with carnosine (prepared by a reaction of one mole of Cr with five or three moles of carnosine under different experimental conditions (reaction time and temperature as well as slightly acidic, neutral or slightly alkaline medium).

In *in vitro* experiments (on total glucose uptake into L6 muscle cells at 100 nmol concentration), CRC5 increases on average 3.57, CRC3R 4.00 and CRC3V 4.45 fold over control.

In vivo experiments gave much more dramatic differences:

TABLE 1

Diabetic Rats Treated with	**Lambda $_{max}$**	**Fasted Blood Glucose Fold Increase over the Starting Point After 4 weeks)**
Cr-picolinate	504 nm	2.37 (95.56%)
Chromex	569 nm	1.88 (75.80%
CRC 5	531.5 nm	2.15 (86.70%)
CRC 3R	540.5 nm	1.74 (70.16%)
CRC 3V	558 nm	1.09 (43.95%)
Untreated		2.48 (100%)



Based on the data from table 1, it can be concluded that there is a clear relationship between the lambda max of a Cr complex (i.e., between the strength of the Cr-Ligand Coordination Bond) and its biological activity. The optimal range for an absorption maximum is around 560 nm. It is well known that stronger coordination bonds produce more stable complexes that have lower values of lambda max, while weaker coordination bonds produce less stable complexes that show absorption maxima at higher nm values.

In other words, the stability of a Cr complex is its key chemical/biochemical property that directly reflects onto the biological activity of the same.
If complexes are too stable (Cr picolinate, for example) activity is low, and the same is true if the complexes are very unstable.
That is to say, stable complexes look to be rather bioavailable but once they reach the target cells they do not readily transfer Cr atom to the Cr-specific binding protein(s).
On contrary, week complexes are unstable and they are not sufficiently bioavailable (decomposing in digestive tract into insoluble Cr-hydroxide that is not bioavailable at all.

Cr complexes of an intermediate stability are both bioavailable and active in target cells since they transfer their Cr ion faster and easier than strong complexes would do.

Another important point is water solubility of a Cr complex. Cr picolinate, Cr niacin and Cr yeast are all water insoluble chemical entities and thus their bioavailability by definition is diminished. Our new Cr complexes are all water soluble, sufficiently stable and bioavailable.

As an example, we mention here our new Cr complex ("Chromex") that is made by a reaction of water soluble brewer's yeast extract and Cr chloride hexahydrate. A typical preparation includes a step in which yeast (*e.g.*, baker's yeast) is grown to a desired density, optionally washed and harvested. The cells are then preferably lysed, and the lyaste is cleared (*e.g.*, via centrifugation, filtration, etc.) to produce an aqueous yeast extract. This extract may further be processed (*e.g.*, to remove nucleic acids, or protein, add amino acids, or other modification) or used as prepared in another step where the extract is combined with a nutritionally acceptable ion (here: CrIII; *e.g.*, as CrCl3 salt, or solution thereof). It is contemplated that the ion will form numerous chemically distinct complexes (*e.g.*, with peptides, lipoproteins, polysaccharides, etc.), and that such complexes are sufficiently stable and water soluble, which is superior to so called Cr yeast that is water insoluble material. Leaching organically bound Cr from Cr yeast is an incomplete process and the Cr bioavailability for Cr yeast is seriously diminished.

In other examples, a broader class of water soluble Cr complexes that have appropriate chemical, spectral and biological properties and that can be prepared by reaction a water soluble Cr (III) species with a water soluble organic matrix (yeast extract and/or autolysate, different protein, peptide, amino acid, nucleoside containing natural extracts (like the malted barley extract and the like), different clear fruit juice concentrates, followed by a final freeze drying process. Thus, contemplated complexes exhibit superior solubility in water as well as an improved association constant (range) to provide biologically active complexes from which the ion can be retrieved under physiological conditions

Further examples of appropriate Cr complexes include heterogeneous Cr complexes (binary and ternary Cr complexes) that contain two, three or more different organic and inorganic ligands. The examples of such complexes are given in the appendix: NEW CHROMIUM COMPLEXES. In this appendix the abbreviated names are given together with short procedures of preparing them. Some of these complexes have the optimal spectral characteristics and their biological examination is ongoing. As an example we mention here PK-1C Cr-Kinetin-Citrate and PK-1 (Cr-Kinetin-Chloride). Both complexes absorb at 564 nm and preliminary in vitro data for PK-1 complex gave at 100 nmole concentration 2.3 fold increase (compared to control) of glucose uptake into L6 muscle cells.

In still further aspects, water soluble yeast extracts may also be used to complex ions other than CrIII, and all nutritionally acceptable ions are considered suitable for use herein.

NEW CHROMIUM COMPLEXES

1. PK-1C (Chromium-mono-Kinetin-Citrate)
2. PK-2C (Chromium-di-Kinetin-Citrate)
3. PK-3C (Chromium-tri-Kinetin-Citrate)

4. BAK-1C (Chromium-mono-Benzyl-Adenine-Citrate)
5. BAK-2C (Chromium-di-Benzyl-Adenine-Citrate)
6. BAK-3C (Chromium-tri-Benzyl-Adenine-Citrate)

7. CROA-1C (Chromium-Citrate-Aminooxyacetate)
7a. CROA-1 (Chromium-mono-Aminooxyacetate) – 1 mmol SB
7b. CROA-2 (Chromium-bis-Aaminooxyacetate) – 2 mmol SB
7c. CROA-3 (Chromium-tris-Aminooxyacetate) – 3 mmol SB
7d. CROAK-11 (Chromium-mono-Kinetin-mono-Aminoooxyacetate) – 1mmol SB
7e. CROAK-21 (Chromium-bis-Kinetin-mono-Aminooxyacetate) – no SB
7f. CROAK-22 (Chromium-bis-Kinetin-bis-Aminooxyacetate) – no SB
8. CROX-1C (Chromium-Citrate-Oxamate)
8a. CROX-1 (Chromium-mono-oxamate) 1 SB
8b. CROX-2 (Chromium-bis-oxamate) 2SB
8c. CROX-3 (Chromium-tris-oxamate) 3SB

9. CROC-1 (Chromium-mono-Citrate)
10. CROC-2 (Chromium-di-Citrate)

11.CC-2 (Chromium-di-Carnosinate)
12. CC-3 (Chromium-tri-Carnosinate)
12a **CC-V**iolet (acidic pH)
12b. **CC-R**ed (basic pH)

13. HEX (HromEx) (Chromium-chloride in YEX)

Molecular Weights of Starting Materials:

$CrCl_3$ x $6H_2O$ (CC-hh) = 266

Citric Acid mono-hydrate (CA-mh) = 210

Kinetin (K) = 215

Benzyl-Adenine (BA) = 225

Oxamic Acid (OA) = 89

Aminooxyacetic Acid x ½ HCl (AOA) = 109

Tri-sodium-citrate x $2H_2O$ (TSC-dh)= 294

Carnosine (C) = 226

Sodium Bicarbonate (SB) = 84

PREPARATION PROCEDURES:

General Remarks:

A. Take 1 mmol (266 mg) of $CrCl_3$ hexahydrate (CC-hh) in all cases.

B. Take one mmol (210 mg) of citric acid mono-hydrate (CA-mh) in cases 1-9.

C. The bellow given procedure for PK-1C is general for all Cr preparations (1-12). The only variables are the nature and quantity of applied ligands.

1. **PK-1C**: Mix 266 mg of CC-hh, 210 mg of CA-mh, and 215 mg (1 mmol) of K in six mL of water. Heat in a glass test tube (at boiling water bath for a short time). Add cautiously 168 mg (2 mmol) of SB (CO_2 evolution!). Heat at the boiling water bath for next 2 hours. Transfer the clear blue-violet solution into a plastic vial (14 mL) and dilute to 10 mL (100 mmol solution of PK-1C).

2. **PK-2C**: 266 mg CC-hh, 210 mg CA-mh, 430 mg (2 mmol) of K and *84 mg (1 mmol) of SB*.

3. **Preparation of PK-3C** (Chromium-tri-Kinetin-Citrate)

266 mg CC-hh, 210 mg CA-mh and 645 mg (3 mmol) of K *(No SB was added!) is heated in 40 mL of distilled water at 95°C for two hours. K goes slowly into solution and solution becomes gradually violet. Diluted to 50 mL. Final concentration: 20 mmole. Filter(or decant) from small insoluble part.*

4. **BAK-1C**: 266 mg CC-hh, 210 mg CA-mh, 225 mg (1 mmol) of BA, *168 mg SB.*

5. **BAK-2C**: 266 mg CC-hh, 210 mg CA-mh , 450 mg (2 mmol) of BA and *84 mg SB.*

6. **BAK-3C**: 266 mg of CC-hh, 210 mg CA-mh and 675 mg (3 mmol) of BA

7. **CROA-1C**: 266 mg of CC-hh, 210 mg CA-mh, 109 mg of AOA (1mmol) and 252 mg (3mmol) of SB (add in protions cautiously!)

7a. CROA-1: 266 mg CC-hh, 109 mg AOA and 84 mg mg SB in ~ 10 mL of water. Heat at 95 C for two hours. Dilute to 10 mL exactly.

7c. CROA-3: 266 mg CC-hh, 32 mg AOA and 252 mg SB in ~ 10 mL of water. Heat at 95 C for two hours. Dilute to 10 mL exactly.

7f. CROAK-22: 266 mg CC-hh, 218 mg AOA, and 430 mg K in ~ 10 mL of water. Heat at 95 C for two hours. Dilute to 10 mL exactly.

D. **CROX-1C**: 266 mg of CC-hh, 210 mg CA-mh, 89 mg OA, and 252 mg SB (see 7.). CROX-8a – 8c (work out the synthetic procedures)

E. **CROC-1**: 266 mg of CC-hh and 294 mg of TSC-dh (1mmol)

F. **CROC-2**: 266 mg of CC-hh and 588 mg of TSC-dh (2 mmol)

G. **CC-2**: 266 mg CC-hh, 452 mg (2 mmol) C and 168 mg of SB (2mmol)

H. **CC-3**: 266 mg CC-hh, and 678 mg (3 mmol) C.

Three Different Procedures:

a) Dissolve CC-hh in 3 ml water; Dissolve C in three ml water; Mix quickly. Precipitate forms; Heat for a short time and add slowly 252 mg SB. Heat 2 hours at boiling wather bath. Appreciable amount of precipitate stays insoluble on prolonged heating. It looks like Cr-hydroxide. Final pH Basic.

b) Dissolve CC-hh in 6 ml water. Heat at the boiling water bath for a short time. Add slowly to hot solution solid C. No precipitate forms. Heat for another 10 minutes and then add slowly and cautiously 160 mg SB. No precipitate froms. On prolonged heating (two to three hours) the solution satys clear. The final color is in between red and violet, closer to red. It is named: **CC-3R (12b)**. Final pH close to neutral.

c) **Dissolve CC-hh in water (6 ml). Heat and add slowly C. No precipitate! Do not add SB!! Heat for next 2 hours at the boiling water bath. Stays clear! Stays slightly acidic. Color: Violet. It is named: CC-3V (12a).**

I. **HEX**: 266 mg CC-hh, 400 mg (AMBEREX)) in 10 mL water. Keep 2 hours at RT. Filter from small insoluble discarded part.

REMARK:

12 a: CC-3 Violet, water soluble (the most acidic pH). Taken for animal experiment at Ray's Lab. 13., HEX, taken for animal experiment. 1., PK-1C, taken for animal experiment.

12 b : CC-3 Red(-violet), water soluble (pH close to neutral). Should be compared to CC-3V in the second experiment with Ray M.

Compounds in "violet font" are tested in the first experiment (at Ray's Lab, on streptozocin-induced diabetes rats).

All compounds in "blue font" will be tested in vitro by ZB. In addition, CC-3R will be tested in second experiment with Ray.

Total glucose uptake in L6 muscle cells in vitro induced by various chromium compounds. Summary of screening results

San Diego, September 08, 2003

Experiments were performed in FutureCeuticals, Inc., 5080 Shoreham PL, Ste 205, San Diego, CA 92122.

Compounds	Conc. nM	Fold over Control	Range	Average
CrCl3	10	1.44, 1.23, 0.95, 3.38, 0.94, 1.50, 1.44, 2.1, 1.3, 1.34, 1.46	0.94-3.38	1.55
	100	1.46, 1.33, 4.05, 2.0, 2.44, 1.56, 2.30, 1.52, 1.93	1.33-4.05	2.05
	1000	1.64, 1.24, 3.05, 2.83, 2.76. 1.68, 1.60, 1.63, 2.42	1.24-3.05	2.08
CRX/Hex	10	4.44, 1.66, 3, 1.70, 2.40, 1.50, 1.40, 1.51	1.40-4.44	2.15
	100	4.88, 3.94, 2.75, 2.01, 4.50, 2.40, 1.84, 2.29	1.84-4.88	3.07
	1000	3.83, 4.26, 4.10, 2.44, 3.90, 3.3 2.39, 1.94	1.94-4.26	3.26
CRC3R	10	3.5, 3.0, 2.5, 1.6	1.6-3.5	2.65
	100	4.5, 4.0, 4.5, 3.0,	3.0-4.5	4.00
	1000	6.0, 2.60, 3.20, 2.0,	2.0-6.0	3.45
CRC3V	10	4.70, 1.88, 2.0, 3.0, 1.71	1.71-4.70	2.65
	100	8.50, 2.20, 4.9, 2.2,	2.20-8.50	4.45
	1000	4.10, 2.90, 3.00, 3.40, 2.10	2.10-4.10	3.10
CRC5	10	2.50, 1.77, 1.60, 1.30	1.30-2.50	1.73
	100	3.17, 6.10, 3.2, 2.10,	2.10-6.10	3.57
	1000	3.20, 4.50, 2.60, 2.60	2.60-4.50	3.22
PK-1	10	1.15, 1.60, 1.56, 1.39, 1.30, 3.88, 2.88	1.15-3.88	1.96
	100	1.28, 1.60, 1.71, 1.98, 2.0, 5.10	1.28-5.10	2.28
	1000	1.61, 1.51, 1.92, 1.63, 1.83, 2.27,	1.51-2.27	1.79
PK3	10	1.26, 1.39, 1.49, 1.40, 2.36, 1.50, 1.38	1.26-2.36	1.54

	100	1.50, 1.0, 2.42, 3.45, 2.83	1.0-3.45	2.24
	1000	1.38, 1.0, 1.50, 2.37, 3.33	1.0-3.33	1.91
CROA -1	10	1.41, 1.66, 2.10,	1.41-2.10	1.72
	100	2.12, 2.16, 2.80,	2.12-2.80	2.36
	1000	2.58, 2.10, 2.50	2.10-2.58	2.39
CROA-1C	10	1.08, 1.07, 1.44,	1.07-1.44	1.19
	100	1.32, 1.35, 1.90	1.32-1.90	1.52
	1000	1.48, 1.96, 2.06	1.48-2.06	1.83
CROA-3	10	1.46, 1.16, 1.79	1.16-1.79	1.47
	100	1.78, 1.38, 1.78	1.38-1.78	1.64
	1000	2.12, 1.75, 2.02,	1.75-2.12	1.96
OA-K1	10	1.98, 3.0, 1.96, 1.72, 2.20	1.72-3.00	2.16
	100	2.52, 3.07, 2.68, 2.28, 1.99	1.99-3.07	2.50
	1000	2.07, 2.09, 2.60, 2.52, 1.60	1.60-2.60	2.17
MTF	10uM	2.16, 2.30, 2.80, 2.26	2.16-2.80	2.38
	100	3.59, 5.0, 3.0, 3.0, 2.0, 2.24, 2.57	2.0-5.0	3.10
	1000	2.0, 2.45, 2.04	2.04-2.45	2.16

Method: Total glucose uptake was measure using fluorescent analog of glucose, 2-NBDG from Molecular Probes Inc. L6 myoblastic cells were treated for 2 hrs with tested compounds in culture medium SkBM from Clonetics. After washing, cells were transferred to HBSA (Hepes-buffered Saline), pH 7.0 with 50uM of 2-NBDG without glucose. One minute later, cells were washed with ice-cold PBS, and fixed in -20C 70% ethanol. Fluorescence was measured at 480/530 (excitation/emission).

Activity of selected chromium compounds in vivo.

Model: Streptozocin-induced insulin deficient rats (commonly known as streptozocin – induced diabetes type I rats).

Steptozocin causes damage of pancreas resulting in drastically reduced secretion of insulin. As consequence, these rats develop severe hyperglycemia.

So far, chromium was known to potentate action of insulin, regardless that exact mechanism was not known. Recently, chromium was found to stimulate AKT thus inducing glucose uptake to muscle cells in insulin-independent way. Still, activation of AKT may justified why chromium was recognized for long time as insulin enhancer.

In our studies in vivo, chromium compounds were provided in drinking water for four weeks at dose 42ug/kg. Vein blood was collected following four hrs fasting and used for fasted blood glucose level test.

Preliminary results from two independent studies in vivo

Study I

Compound	Fasted Blood Glucose Level [Fold increase over 4 weeks]
Untreated	1.95
CrCl3	1.32
CRC3-V	0.80
CRC 5	1.02
CrPic	2.02
Chromex	1.55
CrNiacin	1.91
Metformin	0.96

Study II*

Untreated	2.48
CrPic	2.37
Chromex	1.88
CRC 3-R	1.74
CRC3-V	1.09
CRC5	2.15

* study is still ongoing and results are based on three weeks of treatment only

These results show quite dramatic improvement of glucose transport in insulin-deficient rats. These rats are hypo-insulinemic and hyper-glycemic due to severe pancreatitis conditions. Improvement under such conditions indicate that the treatment overpass insulin-deficiency and stimulate glucose utilization.

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